

pAntiBA 3'-(P)CCTACACGACGTTCCGCTAA (F)-5' (SEQ ID NO: 2)

A' *cancel*

pBA and pAntiBA were synthesized and HPLC purified by Genset Corporations (La Jolla, CA). pAntiBA20 was labeled with Fluorecein at 5'-end, and blocked with a phosphate group at its 3'-end. The relative orientation of the primers (above) are arranged to facilitate viewing of how they will hybridize to each other. 25 µl reactions contained 200 nM ROX-ddC, 4 U polymerase, 250 nM pBA 250 nM pAntiBA, and 200 nM pBluescript in 1 x polymerase reaction buffer. Negative control lacked DNA template (pBluescript). Thermal cycling was performed in the Applied Biosystems Prism 7700 Sequence Detector. Thermal cycling conditions were performed by initial denaturing step at 95°C for 2 minutes, followed by 30 cycles at 95°C for 30 s, 50°C for 1 min, and 57°C for 30 s. The fluorescent intensities were acquired during the annealing/extension phase of the primer extension cycles. The analysis was done using the multicomponent data from the Applied Biosystems 7700 Sequence Detector. Figure 4 illustrates that the positive control (A4 well) shows a ROX signal increase due to FRET from Fluorescein compared to the negative control (A3 well).--

Please delete the paragraph at page 56, line 22 through line 26, to page 57, line 1 through line 17.

Please replace the paragraph at page 56, lines 22 through 26, to page 57, line 1 through line 17 with the following paragraph:

- -Example 2. Detection Of Nucleotide At Predetermined Position Using a probe partially complementary to the primer

A 2

Detection of SNPs was also performed by FRET minisequencing using a probe which is partially complementary to the primer. The primer pJ was designed to anneal to pBluescript (A562C) so that the dideoxynucleotide to be incorporated is a ddCTP.

pJ 5'- GAGGCTCGGAGCGGTTAAACGGATGTGCTGCAAGGCGATT -3' (SEQ ID NO: 3)

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|---|
| |
| <div style="display: inline-block; width: 45%; text-align: center;">Non-complementary to template</div> <div style="display: inline-block; width: 45%; text-align: center;">Complementary to template</div> |

pAntiJ 3'-(P)CTCCGAGCCTCGCCAATTTG(F)-5' (SEQ ID NO. 4)

A2
conc'l

Oligos were synthesized and HPLC purified by Genset Corporations (La Jolla, CA). pAntiJ was labeled with Fluorecein at 5'-end, and blocked with a phosphate group at its 3'-end. The relative orientation of the primers (above) are arranged to facilitate viewing of how they will hybridize to each other. 25 µl reactions contained 200 nM ROX-ddC, 4 U polymerase, 250 nM pJ 250 nM pAntiJ, and 200 nM pBluescript in 1 x polymerase reaction buffer. Negative control lacked DNA template (pBluescript). Thermal cycling was performed in the Applied Biosystems Prism 7700 Sequence Detector. Thermal cycling conditions were performed by initial denaturing step at 95°C for 2 minutes, followed by 30 cycles at 95°C for 30 s, 50°C for 1 min, and 57°C for 30 s. The fluorescent intensities were acquired during the annealing/extension phase of the primer extension cycles. The analysis was done using the multicomponent data from the Applied Biosystems 7700 Sequence Detector. Figure 5 illustrates that the positive control (B2 well) shows a ROX signal increase due to FRET from Fluorescein compared to the negative control (B1 well).- -

Please ~~delete~~ the paragraph at page 57, line 18 through 25.

Please ~~replace~~ the paragraph at page 57, line 18 through 25, with the following paragraph:

- -Example 3. Detection Of Nucleotide At Predetermined Position Using a quencher molecule and a probe partially complementary to the primer

A3

Detection of SNPs was also performed by FRET minisequencing using a probe containing a quencher (Figure 6). The primer pJ was designed to anneal to pBluescript (A562C) so that the dideoxynucleotide to be incorporated is a ddCTP.

pJ 5'- GAGGCTCGGAGCGGTAAACGGATGTGCTGCAAGGCGATT -3' (SEQ ID NO: 3)

Non-complementary to
template
Complementary to
template

pAntiJ-BHQ 3'- (P)CTCCGAGCCTCGCCAATTTG(BHQ2) -5' (SEQ ID NO: 4)- -

Amendments to the specification are indicated in the attached "Marked Up Version of Amendments" (pages i - iii).